30 Rec'd PCT/PTO 25 FEB 2002

FORM PTO-1390 (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371

ATTORNEY'S DOCKET NUMBER: MERCK 2386

U.S. APPLICATION NO. (If known, see 37 CFR §1.5): 10/069285

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/08059	18 AUGUST 2000	24 AUGUST 1999

TITLE OF INVENTION

METHOD FOR ISOLATING AND PURIFYING GRASS POLLEN ALLERGENS

APPLICANT(S) FOR DO/EO/US

SUCK, Roland, et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ■ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ■ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR §1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/069285	PCT/EP00/08059	MERCK 2386

17. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)):

Search Report has been prepared by the EPO or JPO	$890.00
International preliminary examination fee paid to USPTO (37 CFR §1.482)	$710.00
No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2))	$740.00
Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO	$1040.00
International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $890.00

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	8 - 20 =	0	x $ 18.00	$0.00
Independent claims	1 - 3 =	0	x $ 84.00	$0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 280.00	

TOTAL OF ABOVE CALCULATIONS = $890.00

Reduction of 1/2 for filing by small entity, if applicable. A Verified Small Entity Statement must also be

SUBTOTAL = $890.00

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(f)).

TOTAL NATIONAL FEE = $890.00

Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). $40.00 per property.

TOTAL FEES ENCLOSED = $890.00

Amount to be refunded:

charged:

a. ■ A check in the amount of $890.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 13-3402 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3402. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Customer Number 23,599

23599
PATENT TRADEMARK OFFICE

Filed: 25 FEBRUARY 2002
AJZ:kmo

SIGNATURE

Anthony J. Zelano
NAME

27,969
REGISTRATION NUMBER

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No.	:	PCT/EP00/08059
International Filing Date	:	18 AUGUST 2000
Priority Date(s) Claimed	:	24 AUGUST 1999
Applicant(s) (DO/EO/US)	:	SUCK, Roland, et al.

Title: METHOD FOR ISOLATING AND PURIFYING GRASS POLLEN ALLERGENS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend as follows:

IN THE CLAIMS:

3. (Amended) Method according to Claim 1, characterized in that the extraction is carried out by means to tris/HCI-buffered aqueous solution.

4. (Amended) Method according to Claim 1, characterized in that, in a first step, the group 1, 2, 3, 10 and 13 grass allergens are separated off from other constituents by means of hydrophobic interaction chromatography.

7. (Amended) Method for the in-vivo and in-vitro diagnosis of pollen allergies using the allergens obtained in accordance with Claim 1.

8. (Amended) Pharmaceutical preparation comprising one or more allergens obtained in accordance with Claim 1 and corresponding assistants and excipients.

REMARKS

The purpose of this Preliminary Amendment is to eliminate multiple dependent claims in order to avoid the additional fee. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "**Version With Markings to Show Changes Made**".

Respectfully submitted,

#32,004

Anthony J. Zelano, Reg. No. 27,969
Attorney for Applicants
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza 1
2200 Clarendon Boulevard, Suite 1400
Arlington, VA 22201
Direct Dial: 703-812-5311
Facsimile: 703-243-6410
Email: zelano@mwzb.com

AJZ:kmo

Filed: 25 FEBRUARY 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 3, 4, 7 and 8 were amended as follows:

3. (Amended) Method according to Claim 1 ~~or 2~~, characteriszed in that the extraction is carried out by means to tris/HCI-buffered aqueous solution.

4. (Amended) Method according to ~~one of~~ Claims 1 ~~or 3~~, characteriszed in that, in a first step, the group 1, 2, 3, 10 and 13 grass allergens are separated off from other constituents by means of hydrophobic interaction chromatography.

7. (Amended) Method for the in-vivo and in-vitro diagnosis of pollen allergies using the allergens obtained in accordance with Claims 1 ~~to 6~~.

8. (Amended) Pharmaceutical preparation comprising one or more allergens obtained in accordance with ~~one of~~ Claims 1 ~~to 6~~ and corresponding assistants and excipients.

Method for isolating and purifying grass pollen allergens

The invention relates to a method for the rapid and effective isolation and purification of five allergens from groups 1, 2, 3, 10 and 13 from grass pollen. The natural raw material used for the allergen purification is the pollen of sweet grasses, such as, for example, of *Phleum pratense*. The purification is based on a novel combination of hydrophobic interaction chromatography, gel filtration and cation exchange chromatography. The proteins obtained in this way can be used for improved diagnostics of pollen allergies and for pharmaceutical preparations for the therapy of pollen-allergy diseases.

Type 1 allergies are of worldwide importance. Up to 20% of the population in industrialised countries suffers from complaints such as allergic rhinitis, conjunctivitis or bronchial asthma, which are caused by allergens present in the air (aeroallergens), which are released by various sources, such as plants, mites, cats or dogs. Up to 40% of these type 1 allergy sufferers in turn exhibit specific IgE reactivity in the case of grass pollen allergens (Freidhoff et al., 1986, J Allergy Clin Immunol 78, 1190-201).

The substances which trigger type 1 allergy are proteins, glycoproteins or polypeptides. After uptake via the mucous membranes, these allergens react with the IgE molecules bonded to the surface of mast cells in sensitised persons. If two or more IgE molecules link up with one another through an allergen, this results in the secretion of mediators (for example histamine, prostaglandins) and cytokines by the effector cell and thus in the corresponding clinical symptoms.

Depending on the relative frequency of the allergy sufferers having IgE antibodies against certain allergens, a distinction is made between major and minor allergens. In the case of timothy grass (Phleum pratense), Phl p 1 (Petersen et al., 1993, J. Allergy Clin. Immunol. 92, 789-796),

Phl p 5 (Matthiesen and Löwenstein, 1991, Clin. Exp. Allergy 21, 297-307; Petersen et al., 1992), Phl p 6 (Petersen et al., 1995, Int. Arch. Allergy Immunol. 108, 49-54) and Phl p 2/3 (Dolecek et al., 1993) have hitherto been characterised as major allergens and Phl p 4 (Löwenstein, 1978, Prog. Allergy 25, 1-62) and groups 10 and 11 from Lolium perenne (Ansari et al., 1987, J. Allergy Clin. Immunol. 80, 229-235) as minor allergens. In addition, a further high-molecular-weight major allergen that has been named Phl p 13 has recently been described. The group 1 and 13 allergens are glycosylated.

In connection with the present invention, allergy groups 1, 2, 3, 10 and 13 are of particular importance. Established purification methods of the natural allergens are based on isolation of individual proteins in each case. By means of affinity chromatography using specific antibodies, group 1 allergens from *Lolium perenne* (Boutin et al., 1996, Int. Arch. Allergy Immunol. 112, 218-225) and *Phleum pratense* (Grobe et al., 1999, Eur. J. Biochem. 263, 33-40), for example, have been purified hitherto. This method is of limited capacity and is carried out using an extreme pH, with the consequence that it is not guaranteed that the native conformation can be obtained. Other methods are based on various multi-stage sequences of chromatographic steps. Individual allergens, such as, for example, group 10 (Ansari et al., 1987, J Allergy Clin Immunol 80, 229-235) or group 3 (Ansari et al., 1989, Biochemistry 28, 8665-8670), are in each case obtained here. Other allergens are lost or cannot be prepared in pure form in these methods.

DNA sequence data are available, inter alia, for Phl p 1 (Laffer et al., 1994, J. Allergy Clin. Immunol. 94, 1190-98; Petersen et al., 1995, J. Allergy Clin. Immunol. 95 (5), 987-994), Phl p 5 (Vrtala et al., 1993, J. Immunol. 151 (9), 4773-4781), Phl p 6 (Petersen et al., 1995, Int. Arch. Allergy Immunol. 108 (1), 55-59) and Phl p 2 (Dolecek et al., 1993, FEBS 335 (3), 299-304). With the aid of cDNA sequences, it is possible to produce recombinant allergens

which can be used in diagnostics and therapy (Scheiner and Kraft, 1995, Allergy 50, 384-391).

A classical approach to effective therapeutic treatment of allergies is specific immunotherapy or hyposensitisation (Fiebig, 1995, Allergo J. 4 (6), 336-339; Bousquet et al., 1998, J. Allergy Clin Immunol. 102 (4), 558-562). In these methods, the patient is injected subcutaneously with natural allergen extracts in increasing doses. However, this method entails the risk of allergic reactions or even anaphylactic shock. In order to minimise these risks, innovative preparations in the form of allergoids are employed. These are chemically modified allergen extracts which have significantly reduced IgE reactivity, but identical T-cell reactivity compared with the untreated extract (Fiebig, 1995, Allergo J. 4 (7), 377-382).

A greater degree of therapy optimisation would be possible with highly purified allergens. Defined cocktails from natural allergens are able to supersede the previous extracts since the latter, in addition to the various allergens, contain a relatively large number of immunogenic, but non-allergenic accompanying proteins which are not necessary for the specific immunotherapy. The use of allergen cocktails also enables the preparation of patient-specific allergen mixtures corresponding to the sensitisation spectrum. Realistic perspectives which could result in safe hyposensitisation with high-purity natural allergens are offered by modified allergens in which IgE epitopes are destroyed by irreversible modification of the secondary and tertiary structure without impairing the T-cell epitopes which are essential for the therapy.

The invention can likewise advantageously be used in in-vitro and in-vivo diagnostics of allergic illnesses, especially of pollinosis. To this end, the purified allergen groups are employed in established methods for the detection of IgE antibodies.

The invention relates to a biochemical purification method which results in the isolation of 4 major allergens and 1 minor allergen from aqueous short-time pollen extracts by an efficient three-stage purification. The natural raw material used is pollen of the Graminae, such as, for example, *Phleum pratense, Lolium perenne, Dactylis glomerata, Poa pratensis, Cynodon dactylon, Holcus lanatus*, inter alia. Fig. 1 shows the purification scheme of the 5 allergens mentioned from grass pollen extracts. The allergen names Phl p 1 to Phl p 13 correspond to the names allergens 1 to 13 otherwise used in the text.

The invention thus relates to a method for isolating essentially pure group 1, 2, 3, 10 and 13 grass allergens in which an aqueous extract of Graminae pollen is prepared from, and the soluble constituents are subjected to hydrophobic interaction chromatography, a gel filtration step and, if desired, cation exchanger chromatography.

In accordance with the invention, it is also possible to carry out a plurality of steps of one type of chromatography, but in general the method is so effective that one separation step is sufficient in each case.

The method is particularly suitable for isolating said allergens from the pollen of the species *Phleum pratense, Lolium perenne, Dactylis glomerata, Festuca pratensis, Holcus lanatus, Poa pratensis, Secale cereale*.

In a preferred embodiment, the extraction is carried out by means of tris/HCl-buffered aqueous solution. However, it is also possible to employ, in accordance with the invention, other known aqueous buffer solutions.

For the purification of the said allergens, the soluble constituents of the extract are employed. To this end, the extract is centrifuged for from 3 to 8 minutes, preferably 5 minutes, at from 18,000 to 30,000 x g, and the super-

natant is taken for further purification. Alternatively, the insoluble constituents can be separated off by other methods, for example by filtration.

The first chromatographic purification step is carried out by means of hydrophobic interaction chromatography, for example on Sepharose®. In this, a large number of impurities are immobilised on the support, while the desired allergens are located in the fraction passing through the column. Corresponding other support materials can likewise be employed.

The invention thus relates to a corresponding method in which the group 1, 2, 3, 10 and 13 grass allergens are separated off from other constituents by means of hydrophobic interaction chromatography.

In the subsequent purification step, the grass allergens are separated into three fractions, with groups 1 and 13 each representing one fraction and groups 2, 3 and 10 representing the third fraction. The invention thus relates in particular to a method in which the group 1 and 13 allergens are obtained in separate fractions by a subsequent gel filtration step and are separated off from the group 2, 3 and 10 allergens.

The latter can then be separated from one another in accordance with the invention by a subsequent chromatography step via a cation exchanger. The invention therefore relates to a method in which the group 2, 3 and 10 allergens obtained after the gel filtration step are separated from one another by subsequent cation exchange chromatography.

The said allergens, which are known per se, are identified either via their known, different physical, chemical, biological or immunological properties, in particular by means of isoelectric focusing, UV absorption measurements, SDS-PAGE and specific antibodies. These methods and techniques are known and have been described in general terms.

The yield of the allergens obtained in accordance with the invention is 0.5 – 1.5%, based on the originally employed total protein of the grass pollen.

The invention also serves to improve in-vivo and in-vitro diagnostics as part of identification of the patient-specific sensitisation spectrum which resolves allergen components. The invention thus relates to methods for the in-vivo and in-vitro diagnosis of pollen allergies using the allergens obtained in accordance with Claims 1 to 6.

The invention likewise serves to prepare improved preparations for specific immunotherapy of grass pollen allergies, which is achieved by separating off extract constituents which are immunogenic, but are irrelevant for the therapy. It is furthermore possible, through chemical reaction of the purified allergens, to obtain an allergoid preparation. The invention thus also relates to a pharmaceutical preparation which comprises one or more allergens obtained by the method according to the invention and, if desired, corresponding assistants and excipients.

The method is described in detail below:

The purification of the natural allergens from timothy grass pollen is carried out in a three-step process (see Fig. 1). After aqueous extraction with tris/HCl-buffered solution (20 mM tris/HCl, 1 mM EDTA, pH 8.0) of pollen for 30 minutes, the extract is separated by centrifugation, preferably at 20,000 x g for five minutes. The tris/HCl-buffered (20 mM tris/HCl, 1 mM EDTA, pH 8.0) supernatant is treated with 1 M ammonium sulfate and subsequently subjected to hydrophobic interaction chromatography (Phenyl-Sepharose High Performance, Pharmacia). A typical column is packed with from 50 to 100 ml of the support material and operated at a flow rate of about 5 ml/min. The fraction passing through the column comprises exclusively the proteins of five allergen groups: group 1 (30-35 kDa), group 2 (11 kDa), group 3 (12 kDa), group 10 (13 kDa) and group 13 (55-60 kDa).

This is followed by a reduction in the volume, preferably by ultrafiltration or lyophilisation.

In a second step, the group 13 and 1 allergens are then separated from the low-molecular-weight allergens by gel filtration in accordance with their different molecular weights using Superdex® 75 prep grade (Pharmacia) or similar known support materials which are suitable for this purpose. The elution medium is preferably 50 mM ammonium hydrogencarbonate. The column is operated at a flow rate of about 5 ml/min. Three fractions are obtained which comprise allergens 1 and 13 (each separately) and 2, 3 and 10 (in one fraction).

The low-molecular-weight group 2, 3 and 10 allergens, which were eluted together in the third fraction of the gel filtration, are separated from one another by means of cation exchange chromatography. To this end, the lyophilised sample is taken up in an aqueous buffer, preferably 20 mM phosphate buffer, pH 7.2, and applied to a cation exchanger column equilibrated with this buffer (for example Source S ®). The fraction passing through the column contains the acidic allergen 2. The bound allergens 3 and 10 are eluted one after the other over about 20 column volumes by means of a salt gradient from 0 to 500 mM NaCl. The low-molecular-weight allergen group is thus separated into its individual allergens. Other cation exchanger materials can also be employed in accordance with the invention.

By means of the method according to the invention that is provided and which is distinguished by the specific sequence of the chromatography steps and the choice of chromatography media, the present invention thus facilitates a highly scalable production method for the isolation of a plurality of high-purity, natural grass allergens which is not labour-intensive or time-consuming and which can be implemented technologically. Since the purification methods used are very gentle for proteins, their conformations and

antigeneity are retained. This is a prerequisite for successful diagnostics of allergic illnesses.

Patent Claims

1. Method for isolating essentially pure group 1, 2, 3, 10 and 13 grass allergens, characterised in that an aqueous extract of Graminae pollen is prepared, and the soluble constituents are subjected to hydrophobic interaction chromatography, a gel filtration step and, if desired, cation exchanger chromatography.

2. Method according to Claim 1, characterised in that pollen of the species *Phleum pratense, Lolium perenne, Dactylis glomerata, Festuca pratensis, Holcus lanatus, Poa pratensis, Secale cereale* are used for the extraction.

3. Method according to Claim 1 or 2, characterised in that the extraction is carried out by means of tris/HCl-buffered aqueous solution.

4. Method according to one of Claims 1 to 3, characterised in that, in a first step, the group 1, 2, 3, 10 and 13 grass allergens are separated off from other constituents by means of hydrophobic interaction chromatography.

5. Method according to Claim 4, characterised in that the group 1 and 13 allergens are obtained in separate fractions by a subsequent gel filtration step and are separated off from the group 2, 3 and 10 allergens.

6. Method according to Claim 5, characterised in that the group 2, 3 and 10 allergens obtained after the gel filtration step are separated from one another by subsequent cation exchange chromatography.

7. Method for the in-vivo and in-vitro diagnosis of pollen allergies using the allergens obtained in accordance with Claims 1 to 6.

8. Pharmaceutical preparation comprising one or more allergens obtained in accordance with one of Claims 1 to 6 and corresponding assistants and excipients.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
1. März 2001 (01.03.2001)

PCT

(10) Internationale Veröffentlichungsnummer
WO 01/13946 A2

(51) Internationale Patentklassifikation[7]: A61K 39/36

(21) Internationales Aktenzeichen: PCT/EP00/08059

(22) Internationales Anmeldedatum:
18. August 2000 (18.08.2000)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 39 982.4 24. August 1999 (24.08.1999) DE

(71) Anmelder *(für alle Bestimmungsstaaten mit Ausnahme von US)*: MERCK PATENT GMBH [DE/DE]; Frankfurter Strasse 250, D-64293 Darmstadt (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: SUCK, Roland [DE/DE]; Mühlenkamp 19, D-22303 Hamburg (DE). CROMWELL, Oliver [DE/DE]; Loenshöhe 2, D-21465 Wentorft (DE). FIEBIG, Helmut [DE/DE]; Bäckerweg 10, D-21493 Schwarzenbek (DE).

(74) Gemeinsamer Vertreter: MERCK PATENT GMBH; Frankfurter Strasse 250, D-64293 Darmstadt (DE).

(81) Bestimmungsstaaten *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Bestimmungsstaaten *(regional)*: ARIPO-Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht:
— *Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.*

Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) Title: METHOD FOR ISOLATING AND PURIFYING GRASS POLLEN ALLERGENS

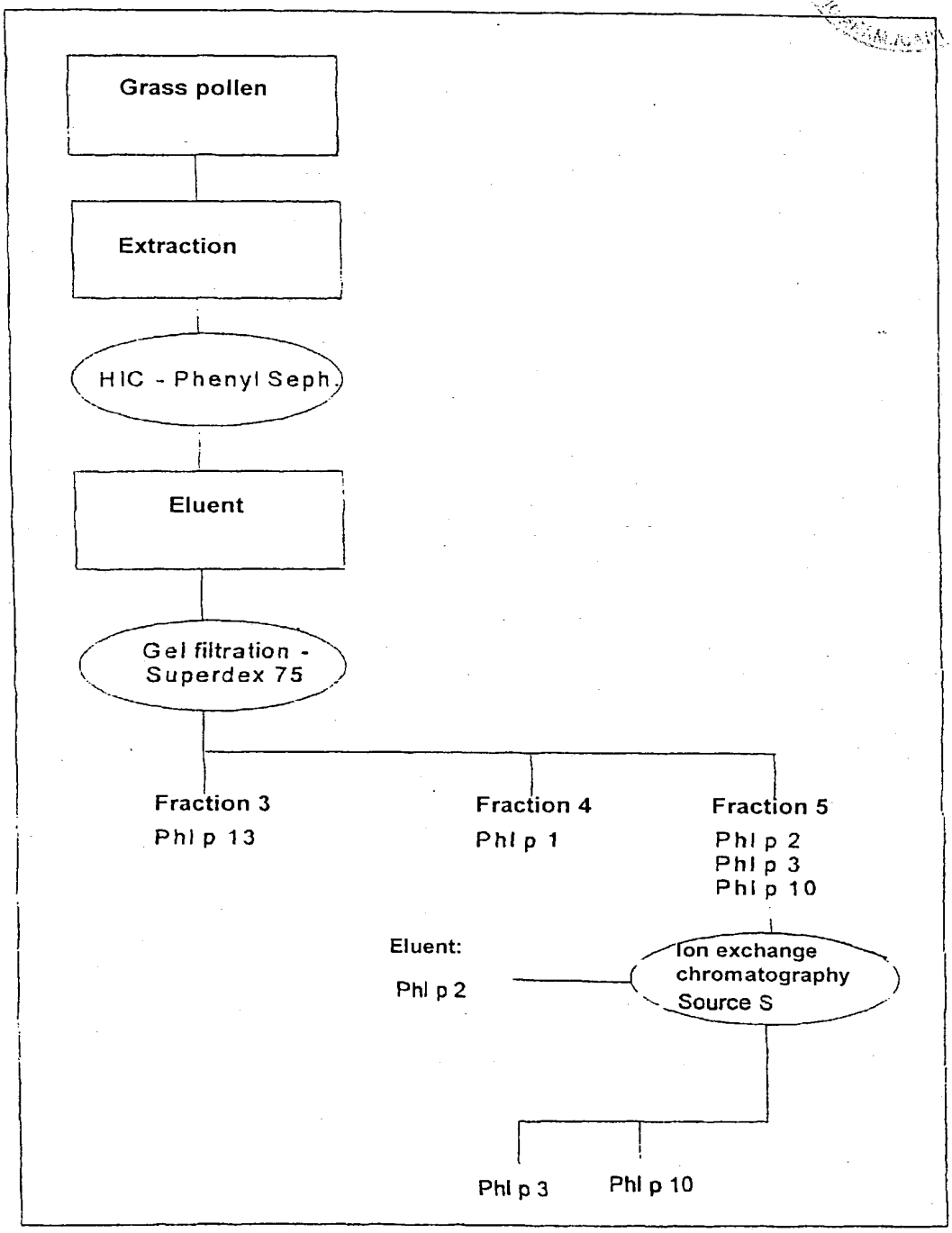
(54) Bezeichnung: VERFAHREN ZUR ISOLIERUNG UND AUFREINIGUNG VON GRÄSERPOLLENALLERGENEN

(57) Abstract: The invention relates to a method for quickly and effectively isolating and purifying five, namely the group 1, 2, 3, 10 and 13 allergens from grass pollen. The purification of said grass pollen is based on the inventive combination of hydrophobic interaction chromatography, gel filtration and cation exchange chromatography. The proteins obtained by the inventive method facilitate an improved diagnosis of pollen allergies and are used in pharmaceutical preparations for the therapy of pollenogenic diseases.

(57) Zusammenfassung: Die Erfindung betrifft ein Verfahren zur schnellen und effektiven Isolierung und Reinigung von fünf Allergenen der Gruppen 1, 2, 3, 10 und 13 aus Gräserpollen. Die Aufreinigung basiert auf einer erfindungsgemäßen Kombination von Hydrophober-Interaktionschromatographie, Gelfiltration und Kationaustauschchromatographie. Die so erhaltenen Proteine können zur verbesserten Diagnostik von Pollenallergien sowie für pharmazeutische Zubereitungen für die Therapie von pollenallergischen Krankheiten verwendet werden.

WO 01/13946 A2

Fig. 1.:


Grass pollen
Extraction
HIC - Phenyl Seph.
Eluent
Gel filtration - Superdex 75
Fraction 3
Phl p 13
Fraction 4
Phl p 1
Fraction 5
Phl p 2
Phl p 3
Phl p 10
Eluent:
Phl p 2
Ion exchange chromatography Source S
Phl p 3
Phl p 10

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
MERCK-2386

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR ISOLATING AND PURIFYING GRASS POLLEN ALLERGENS

the specification of which (check only one item below)

☐ is attached hereto

☒ was filed as United States application

Serial No. 10/069,285

on February 25, 2002

and was amended

on _____ (if applicable)

☒ was filed as PCT international application

Number PCT/EP00/08059

on August 20, 2000,

and was amended under PCT Article 19

on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim priority benefits under Title 35, United States Code, § 119 or 365 (b) of the following United States provisional application(s) and of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

PRIOR U.S. PROVISIONAL AND FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Germany	19939382.4	24/08/99	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

POWER OF ATTORNEY: As a named inventor, I hereby appoint I. William Miller (19,544); John L. White (17,746), Anthony J. Zelano (27,969); Alan E.J. Branigan (20,565), John R. Moses (24,983); Harry B. Shubin (32,004), Brion P. Heaney (32,542), Richard J. Traverso (30,595); John A. Sopp (33,103); Richard M. Lebovitz (37,067), James E. Ruland (37,432); Nancy Axelrod (44,014); Jennifer J. Branigan (40,921), Robert E. McCarthy, (46,044); and Csaba Henter (50,908) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Send Correspondence to Customer No. 23599

Telephone No 703/243-6333

Direct Telephone Calls to

23599
PATENT TRADEMARK OFFICE

Combined Declaration for Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER: MERCK-2386

201	FULL NAME OF INVENTOR	FAMILY NAME: SUCK	FIRST GIVEN NAME: Roland	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: Hamburg	STATE OR FOREIGN COUNTRY: Germany DEX	COUNTRY OF CITIZENSHIP: Germany
	POST OFFICE ADDRESS	STREET: Mühlenkamp 19	CITY: Hamburg	STATE & ZIP CODE/COUNTRY: D-22303 Germany
202	FULL NAME OF INVENTOR	FAMILY NAME: CROMWELL	FIRST GIVEN NAME: Oliver	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: Wantorf	STATE OR FOREIGN COUNTRY: Germany DEX	COUNTRY OF CITIZENSHIP: Germany
	POST OFFICE ADDRESS	STREET: Loenschoche 2	CITY: Wantorf	STATE & ZIP CODE/COUNTRY: D-21465 Germany
203	FULL NAME OF INVENTOR	FAMILY NAME: PIEBIG	FIRST GIVEN NAME: Helmut	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: Schwarzenbek	STATE OR FOREIGN COUNTRY: Germany DEX	COUNTRY OF CITIZENSHIP: Germany
	POST OFFICE ADDRESS	STREET: Backerweg 10	CITY: Schwarzenbek	STATE & ZIP CODE/COUNTRY: D-21493 Germany
204	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
	POST OFFICE ADDRESS	STREET:	CITY:	STATE & ZIP CODE/COUNTRY:
205	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
	POST OFFICE ADDRESS	STREET:	CITY:	STATE & ZIP CODE/COUNTRY:
206	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
	POST OFFICE ADDRESS	STREET:	CITY:	STATE & ZIP CODE/COUNTRY:
207	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
	POST OFFICE ADDRESS	STREET:	CITY:	STATE & ZIP CODE/COUNTRY:

1-00

2-00

3-00

Combined Declaration for Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET OR MERC-2386

208	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE & ZIP CODE/COUNTRY
209	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE & ZIP CODE/COUNTRY
210	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE & ZIP CODE/COUNTRY
211	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE & ZIP CODE/COUNTRY
212	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

SIGNATURE OF INVENTOR 201	DATE 8.08.02	SIGNATURE OF INVENTOR 207	DATE
SIGNATURE OF INVENTOR 202	DATE 8. August '02	SIGNATURE OF INVENTOR 208	DATE
SIGNATURE OF INVENTOR 203	DATE 08.08.02	SIGNATURE OF INVENTOR 209	DATE
SIGNATURE OF INVENTOR 204	DATE	SIGNATURE OF INVENTOR 210	DATE
SIGNATURE OF INVENTOR 205	DATE	SIGNATURE OF INVENTOR 211	DATE
SIGNATURE OF INVENTOR 206	DATE	SIGNATURE OF INVENTOR 212	DATE